Vol. 99, No. 3 December, 1950

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

SOME OBSERVATIONS ON A LUMINESCENT FRESHWATER LIMPET FROM NEW ZEALAND

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In view of the statement of Harvey (1940) that ". . . luminous animals are practically entirely marine or terrestrial. No examples of freshwater luminous organisms are known except bacteria and an aquatic glowworm . . .," the luminous properties of the New Zealand freshwater mollusc, *Latia neritoides,* would appear to be a unique and surprising phenomenon. This, together with the fact that, apart from the cephalopods, only three luminescent species of molluscs have been hitherto recorded (Harvey, 1940) is sufficient justification for a full investigation of the apparently exceptional properties of this freshwater limpet.

Latia is a monotypic genus restricted to the North Island of New Zealand. It belongs to the family Ancylidae in which no other luminescent forms have been recorded. The two other New Zealand species (*Gundlachia neozelanica* and *G. lucasi*) are certainly not luminescent.

The luminescence of Latia was first recorded by Suter in 1890 and again referred to in his comprehensive Manual of New Zealand Mollusca (1913). Although the phenomenon is well known to many New Zealanders, its nature and mechanism have never been studied.

It was with this end in view, therefore, particularly in the light of modern researches in the field of bioluminescence, that the present investigation has been undertaken.

Material and Methods

Specimens of *Latia neritoides* were collected near Auckland in December, 1949, and were immediately placed in Bouin's fluid. Subsequently during the investigation, living specimens were obtained in May, 1950, for experimental and histological purposes. It was found that these animals remained alive in the laboratory for more than three weeks if placed in brown glass bottles in the dark, whereas if kept in white glass bottles and exposed to the light they died within a day or two.

In order to contrast the histological structure of Latia with that of an allied nonluminescent form, specimens of *Gundlachia lucasi* were collected locally and submitted to simliar histological procedures.

Luciferin-luciferase reaction. Three animals were shaken in 10 ml. of water in a test-tube at room temperature for 10 minutes. The solution was then decanted into another test-tube and allowed to stand until all luminescence had disappeared, leaving a solution of the thermolabile luciferase. Three more animals were placed in another test-tube with 10 ml. of water at 70° C., crushed, kept at that temperature in a water bath for 10 minutes and then allowed to cool. At 70° C. the thermolabile luciferase is denatured and the substrate, luciferin, is thus obtained unoxidised in solution.

Five ml. of clear solution were then decanted from each tube, neither of which at this stage showed any luminescence. Upon mixing the two samples, however, a brilliant pale green light was produced. This indicated a positive luciferin-luciferase reaction.

The possibility that the presence of "cytolytic" substances in the hot-water solution might cause luminescence, by breaking down residual granules of luciferin in the cold-water extract, was disproved by the method described by Harvey (1940) of adding to the cold water solution several drops of ether which produces a similar effect. No luminescence resulted in this solution, thus proving the absence of residual granules of luciferin, while the presence of the ether did not inhibit the production of light when the two solutions were mixed. This appeared to confirm the conclusion that the reaction is a true luciferin-luciferase oxidation.

Histology. For histological study the animals were fixed in Bouin's fluid. They were embedded in paraffin and sectioned at 10 μ, both longitudinally and transversely. A variety of staining procedures were used: haematoxylin and eosin, Mayer's mucicarmine, and the Azan stain. An attempt was also made to study the innervation of the specific cells by means of the methylene blue technique, but this was unsuccessful and consideration of this aspect of the problem is deferred.

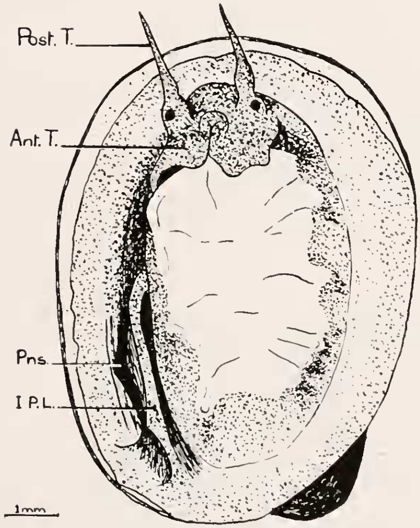
In order to determine the actual source of the luminescent material, several specimens were submitted to prolonged stimulation, by rubbing with a seeker the luminescent zones, in order to exhaust the specific cells. These animals were then submitted to the same histological procedures as before (Figs. 4, 5).

Observations

General. Latia is common in clear streams, often in rapids and other situations where the current is swift; living on the sides or undersurfaces of clean boulders, its limpet shape offers little resistance to the smooth flow of water. It is also found, but much less commonly, in lakes in places where there are clean rocks and considerable water movement, usually very close to the shore. An analysis of the mixed supply of water from the Waitakere Ranges near Auckland indicates its low salt content: sodium chloride was present as 3.04 parts and magnesium chloride as 0.76 parts per 100,000. Latia is abundant in all of these streams and from one of them the specimens used in this investigation were collected.

Like most other luminous forms, Latia shuns strong light and, as already stated, it soon dies if kept exposed to light. It has a well developed eye with the "pupil" directed forwards, and appears to be well adapted to perceive the direction of incident light.

Latia commonly reaches a length of 8.5 mm., a breadth of 6 mm. and a height of 3 mm. Its shell has a thin calcareous layer covered by a stout dark brown periostracum, smooth except for growth lines. Posteriorly, there projects forwards (horizontally within the shell) a semicircular shelf or lamella which on the right side is prolonged forwards into a free calcareous lingula (Fig. 2) which in the living state supports the medial wall of the pneumostome (the inferior pallial lobe) (Fig. 1). Both the lamella and the lingula give origin to the muscular mass of the foot (Fig. 3) and so fix the shell very firmly to the body. Although probably a great advantage in a swift stream, this nevertheless greatly restricts the mobility of the animal relative to the shell, for when placed on its back it is unable to right itself; Gundlachia, which lacks these processes, is able to perform the manoeuvre easily. The mantle cavity of Latia, like that of some marine Basommatophora (the Siphonariidae, the Gadiniidae), is water filled, but as in the Gadiniidae does not contain a branchia. The skeletal support afforded by the lingula appears to prevent both the closure and the over-dilatation of the pneumostome while directing a stream of water into the mantle cavity to flush it.



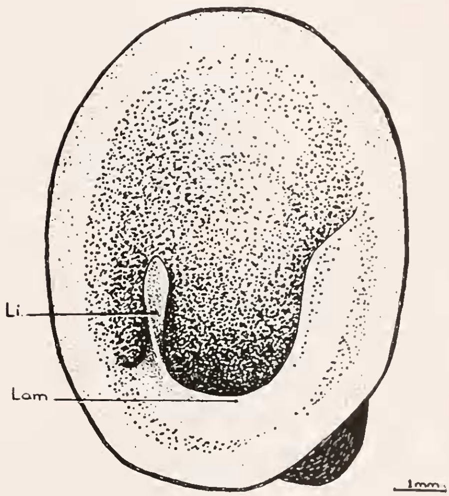


FIGURE 1. *Latia neritoides* Gray. Ventral view of the intact animal. All the parts of the animal that are shown are light producing, except the sole of the foot, the posterior tentacles, and the interior of the pulmonary cavity. Pns., pneumostome. I.P.L., inferior pallial lobe. Ant. T., anterior tentacle, Post. T., posterior tentacle.

FIGURE 2. The interior of the shell showing the semicircular lamella (Lam.) and the lingula (Li.)

Reference may be made to some remarks on the anatomy of Latia by Hutton (1881). Probably because of poorly preserved material he stated that the tentacles were transversely ringed and that the eyes were lateral to the bases of the tentacles. This is obviously not the case either in fresh or fixed specimens (Fig. 1). Hutton also gave the radular formula as $27 + 1 + 27$ but this is certainly wrong, since the number of laterals is approximately 110.

Latia, like the rest of its sub-order, is a vegetarian. The gut is usually filled with diatoms and what appear to be the remains of algae. Similar diatoms are found on the surface of the shell.

The luminescence. Whenever the animal is disturbed, as by shaking in a vessel or tapping the shell, luminous mucus appears in all parts of the groove between the foot and the mantle. This is visible even in diffused daylight as a greenish glow, and in the dark the effect is much more striking. The animals have never been seen to luminesce spontaneously at night either in the laboratory or in their natural habitat, but when they are stimulated they produce the glow equally well during the day or night. Where the mantle groove is touched with a seeker, that region in particular glows most strongly. Shaking the animal in a test tube or rubbing it, foot down, across a glass slide produces trails of glowing mucus from which the light slowly fades.

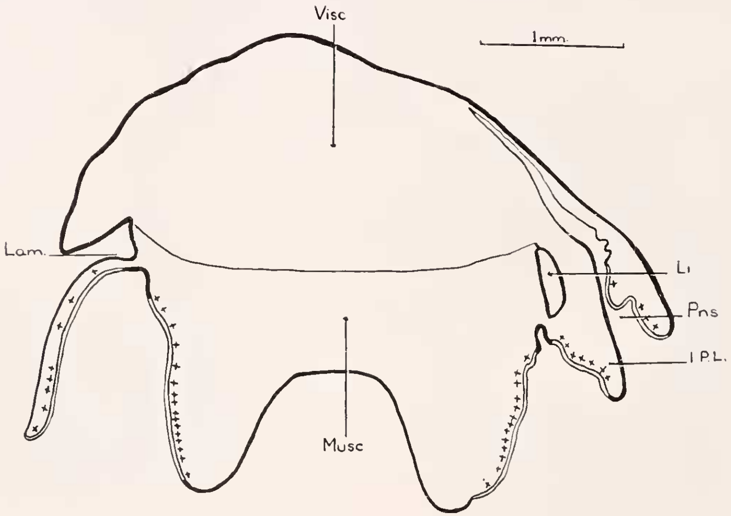
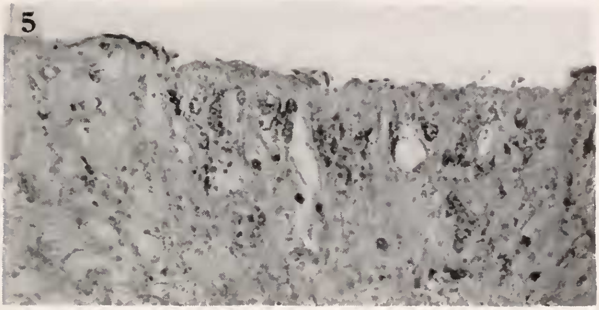


FIGURE 3. Outline of a transverse section (camera lucida). The crosses show the approximate number and distribution of granular cells. (These represent the average distribution in six non-consecutive sections from the same specimen.) Musc., muscular mass of the foot. Visc., visceral hump. (Other letters as for Figs. 1 and 2.)

Clearly then the phenomenon is an extracellular one, and experiments to determine the nature of the luminescence show that it depends on the action of luciferase upon the substrate luciferin, which appear to be produced together from specific areas, particularly along the walls of the mantle groove.

Histology. Examination of the sectioned animal shows that certain parts of its surface present a very striking and characteristic appearance. These regions are the surface of the head, the anterior tentacles, the lateral surfaces of the foot, the inferior pallial lobe, and the free surface of the mantle (but not of the pulmonary cavity). In all these situations, just beneath the simple cuboidal surface epithelium and lying in the loose subepithelial connective tissue, are large numbers of intermingled mucous cells and granular cells, together with branching melanophores and muscle fibres (Figs. 4, 6, 7). Since these regions correspond precisely to those from which the maximum luminous response may be obtained experimentally, they obviously represent the structural apparatus responsible for the luminescence.



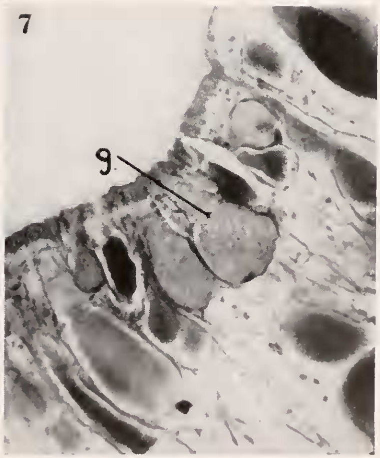


FIGURE 4. General view of luminous epithelium (Haematoxylin and Eosin) × 180.

FIGURE 5. A similar view after the animal had been stimulated to exhaustion (Haematoxylin and Eosin).

FIGURE 6. Two mucous cells in this figure show basally placed nuclei and the large size of the vacuole (Haematoxylin and Eosin) × 250.

FIGURE 7. The granular cell (g) in the center of the figure shows a flattened, basally situated, darkly stained nucleus and granules packing the whole cytoplasm (Azan).

The mucous and granular cells appear to be most probably the specific elements concerned in the phenomenon, since they alone are absent from the non-luminous areas, and they are not found in other non-luminous mollusca including the related Gundlachia.

1. Mucous cells. These are large elongated ovoid cells which may reach a length of 200 μ and a diameter of 40 μ. They taper markedly as they approach the surface epithelium and in many cases their secretion could be observed being extruded at the surface from the apex of the cell. The cells present large swollen vacuoles containing mucus, which stains pink or pinkish-purple with haematoxylin and eosin, purple with Azan, but is not stained by Mayer's mucicarmine. The cytoplasm, which is greatly attenuated, stains blue with Azan but is indistinctly stained with eosin. The nuclei of these distended cells are always basally situated and somewhat compressed.

2. Granular cells. These are elongated cells, rather smaller than the mucous cells (150 $\mu \times$ 30 μ) but like them project into the epidermis. Their cytoplasm is packed with densely basophilic granules which in haematoxylin preparations generally obscure the nucleus. Where it is visible the nucleus is generally, but not always, basally situated and very flattened. With Azan the nuclei stain red, the granules blue.

3. Changes after stimulation. Instead of the large and distinctive mucous and granular cells, which were previously such a striking feature of the subepithelial tissue, it is now difficult to identify any specfiic cells at all. While empty mucous cells, now slender and devoid of secretion, are still recognisable here and there, it is practically impossible to identify with certainty the previously granular cells. There is, in fact, a strong possibility that not only are the entire contents of the granular cells discharged but that the nucleus is extruded as well, in which case mucous cells alone would remain in the exhausted luminescent areas. Whether or not this applies to all the granular cells, it is certainly the case that smears of mucus do contain nuclei of an appearance closely similar to those of the granular cells.

Discussion

The above observations establish quite clearly that Latia is a completely aquatic gasteropod with marked adaptations to life in strong currents; and that its bright luminescence is extracellular and is due to the secretion of luciferin and luciferase.

Harvey (1940) has stated that in only five of the very numerous orders of plants and animals in which luminescence occurs, can the luciferin-luciferase reaction be demonstrated *in vitro*. These include beetles (fireflies), some ostracods, a few polychaete worms, one squid, and now Latia joins *Pholas dactylus* as the second example in the lower mollusca (Plocamopherus and Phyllirrhoë have not yet been tested). Since the only other known example of fresh water luminous animals are the aquatic glowworms, it is interesting to compare Latia with these animals. These glowworms are actually larval coleoptera and have been recorded only from Asia. Annandale (1900) has described one (? Luciola sp.) from Calcutta; another (identified provisionally as *Pyrophanes similis*) was found on the Island of Celebes and described by Blair (1927); while two others from Japan (*Luciola cruciata* and *L. lateralis*) have been described by Okada (1928). Since in these forms, which all possess tracheal gills, the tracheae are full of air, the functioning of the luminescent organs is presumably the same as that in the adults which are terrestrial fireflies. In these, in striking contrast to Latia, the production of light is intracellular and the luminous organs are covered like the rest of the body by a chitinous cuticle (Buck, 1948; Okada, 1928).

The luminous apparatus of Latia is similar to that of certain other luminous invertebrates (Dahlgren, 1915–17), particularly those with a naked epidermis or with only a thin cuticle, such as the coelenterates, polychaetes (especially Chaetopterus), and enteropneusta (Ptychodera). It bears, however, by far the closest resemblance to the luminous apparatus of another mollusc—the peculiar, pelagic opisthobranch *Phyllirrhoë bucephala*, in which both the mucous and the granular cells are almost identical with those of Latia; although I have not observed in Latia the prominent nerve-endings which are described by Dahlgren (1916a) in relation with the granular cells of Phyllirrhoë. In view, however, of the doubt cast on the numerous (and extremely conflicting) descriptions of light-cell innervation in the fireflies (Buck, 1948), the problem of whether or not there is a direct innervation of these cells in mollusca must remain open until such nervous connections have been demonstrated by recognised neurological techniques.

The possibility mentioned above, that the secretion of the granular cells in Latia may be holocrine, seems to be supported by some statements of Dahlgren. He suggests, for instance, that in Chaetopterus the granular cells may be "of a secretory type that are destroyed by one cycle of secretory activity" (1916b); again, when dealing with Pholas (1916a), he recalls Forster's observation that the granules in the granular cells of Pholas fill the cell and appear to be in contact with the nucleus. He comments that this is unusual except in cells which die on discharge of their contents but the mucus does not seem to have been examined for nuclei.

We see, therefore, that although Latia is quite unique among freshwater animals, the actual mechanism of its light-production closely resembles that of certain other marine luminous species, and thus does not bring us any closer to the solution of the very interesting problem: why is it that luminous forms, so common in the sea are so rare in fresh water? It is, however, possible to say now that the reason for their rarity cannot simply depend on a low concentration of salt.

Of the functions which may be attributed to the luminescence of Latia, no positive conclusions can be drawn. It has been suggested that luminescence might subserve three principal purposes not necessarily mutually exclusive: a lure for food; a sexual recognition signal; or a defensive mechanism. In the case of Latia, the first may be dismissed because the animal is a typical herbivore. The second, although it cannot be completely discounted, is unlikely for two reasons: Latia where it occurs is a common species, it is moreover an hermaphrodite and it would seem to have fewer rather than more difficulties to overcome than the nonluminous operculates which occur with it; furthermore its light production is not seasonal and is readily elicited by harmful stimuli.

As for the third possibility: what little evidence there is on the food habits of the fresh water fauna of New Zealand indicates that Latia is eaten by both trout (Phillips, 1929) and eels (Cairns, 1944) but detailed statistics are not available. It seems reasonable to conclude, however, that it is not unpalatable so that the luminescence cannot be equivalent to a warning coloration. A more obscure defensive action has been suggested for other luminous forms by Burkenroad (1943) who has put forward the view that light from specimens attacked by predators might attract secondary predators for the purpose of driving off the primary ones. However the problem is obviously one which cannot be solved until more detailed field work can be undertaken.

In conclusion, it may be stated that the luminescence cannot be regarded as physiological accident or metabolic by-product. The large size and high degree of specialization of the specific cells are themselves sufficient evidence to the contrary. Latia, of course, is not exceptional in lacking an obvious use for its light, for luminous species in which a function is known are the exception rather than the rule (Harvey, 1948).

Acknowledgments

I wish to express my gratitude to Professor W. E. Adams for allowing me to use the laboratories of this Department and for criticising the manuscript; to Mr. A. D. Mead, City Waterworks Engineer, Auckland, for providing the water analysis figures; and to my parents for collecting specimens and sending them to Dunedin.

Summary

1. *Latia neritoides* Gray is a pulmonate freshwater limpet from New Zealand, and is highly adapted to life in streams.

2. It is brilliantly self-luminous due to the extracellular secretion of luciferin and luciferase, and is therefore unique among freshwater animals.

3. The luminous epithelium occurs over much of the animal and is similar in appearance to that found in many marine invertebrates. It is especially similar to that of another gasteropod, Phyllirrhoë. In both species similar mucous and granular cells are found lying in the subepidermal connective tissue and projecting into the epidermis.

4. No definite function can yet be assigned to the light.

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